Changes in neuroendocrine elements in bronchial mucosa in chronic lung disease in adults

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Abstract

***Background* – It is not clear whether there is any association between metaplasia of the bronchial epithelium and changes in the distribution of neuroendocrine cells. This study examined, by immunohistological techniques, the distribution of neuroendocrine cells and juxtamucoscal nerve fibres in bronchial biopsies showing metaplastic changes.**

***Methods* – Bronchial biopsies from 12 subjects with epithelial metaplasia associated with bronchiectasis and diffuse pulmonary fibrosis were examined by conventional light microscopy and immunohistological techniques for protein gene product 9·5 (PGP), chromogranin A and B (CAB), serotonin, vasoactive intestinal peptide (VIP), substance P (SP), calcitonin gene-related peptide (CGRP), calcitonin (CT), and gastrin releasing peptide (GRP).**

***Results* – Regions of non-metaplastic epithelium contained numerous PGP and serotonin immunoreactive cells. Subpopulations of these cells displayed CAB, CGRP, CT, and GRP immunoreactivity. Metaplastic epithelium contained only a few weakly stained PGP, serotonin, CAB, GRP, CT and CGRP immunoreactive cells in six cases. Metaplastic epithelium was characterised by a high number of CAB-containing cells in six cases and in these biopsies prominent PGP-containing nerve bundles were seen in the subepithelial layer beneath the metaplastic epithelium.**

***Conclusions* – The distribution patterns of neuroendocrine cells and neuronal elements vary between areas of normal and metaplastic epithelium and within areas of metaplastic epithelium. Neuronal hyperplasia was associated with an increase in the number of CAB-containing cells within the metaplastic epithelium.**

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Keywords: neuroendocrine cells, metaplasia, bronchial mucosa, chronic non-specific lung disease.

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Elements of the diffuse neuroendocrine system are well represented in the human lung, especially in the large bronchi.[1 2] The density of neuropeptide-containing nerve fibres and the number of pulmonary neuroendocrine cells change only marginally from childhood to old age,[3 4] but the density of cells increases significantly in chronic non-specific lung disease.[5]

It is not known whether there is any association between changes in the occurrence and distribution of neuroendocrine cells and the presence of metaplastic bronchial epithelium. Using immunocytochemical methods we have investigated biopsy samples from 12 patients with chronic non-specific lung disease associated with metaplastic epithelium of large bronchi in order to identify neuroendocrine cells and juxtamucosal nerve fibres. Antisera against the general neuroendocrine markers protein gene product 9·5 (PGP) and chromogranin A and B (CAB), as well as the neurohormonal messengers vasoactive intestinal peptide (VIP), substance P (SP), calcitonin gene-related peptide (CGRP), serotonin, calcitonin (CT), and gastrin releasing peptide (GRP) were used.

Methods

Mucosal biopsy specimens from large bronchi were obtained at bronchoscopy from 12 patients (aged 20–65 years) with bronchiectasis and diffuse pulmonary fibrosis. Patients with bronchiectasis had had the condition for at least 10 years, and four patients with diffuse pulmonary fibrosis for more than 11 months. The biopsy specimens were fixed in a mixture of 2% formaldehyde and 0·2% picric acid in 0·1 M phosphate buffer (pH 7·2). The tissues were then rinsed in a Tyrode buffer containing sucrose. The specimens were frozen on dry ice and sectioned in a cryostat. Sections from each bronchial biopsy specimen were routinely stained with haematoxylin and eosin for light microscopic examination of epithelial metaplasia. For immunocytochemistry[6] we used antisera against the following substances: PGP (rabbit polyclonal, working dilution 1:1600, Ultraclone, Cambridge, UK); serotonin (rabbit polyclonal, 1:1600, Inc Star, Stillwater, USA); CAB (rabbit polyclonal antiserum demonstrating both chromogranins,[7] 1:640, Milab, Malmö, Sweden); chromogranin A (mouse monoclonal, 1:320, Boehringer Mannheim, Germany); VIP (rabbit polyclonal, 1:640, Milab, Malmö, Sweden), CGRP (rabbit polyclonal, 1:1280, Milab, Malmö, Sweden); SP (rabbit polyclonal, 1:320, gift of Dr PC Emson, MRC, Cambridge, UK); calcitonin (rabbit polyclonal, 1:640, Milab, Malmö, Sweden); GRP (rabbit polyclonal 1:640, gift of Professor N Yanaihara, Shizuoka, Japan). The specimens were incubated overnight with primary antiserum at +4°C. After thorough rinsing in phosphate buffered saline (PBS) the sections

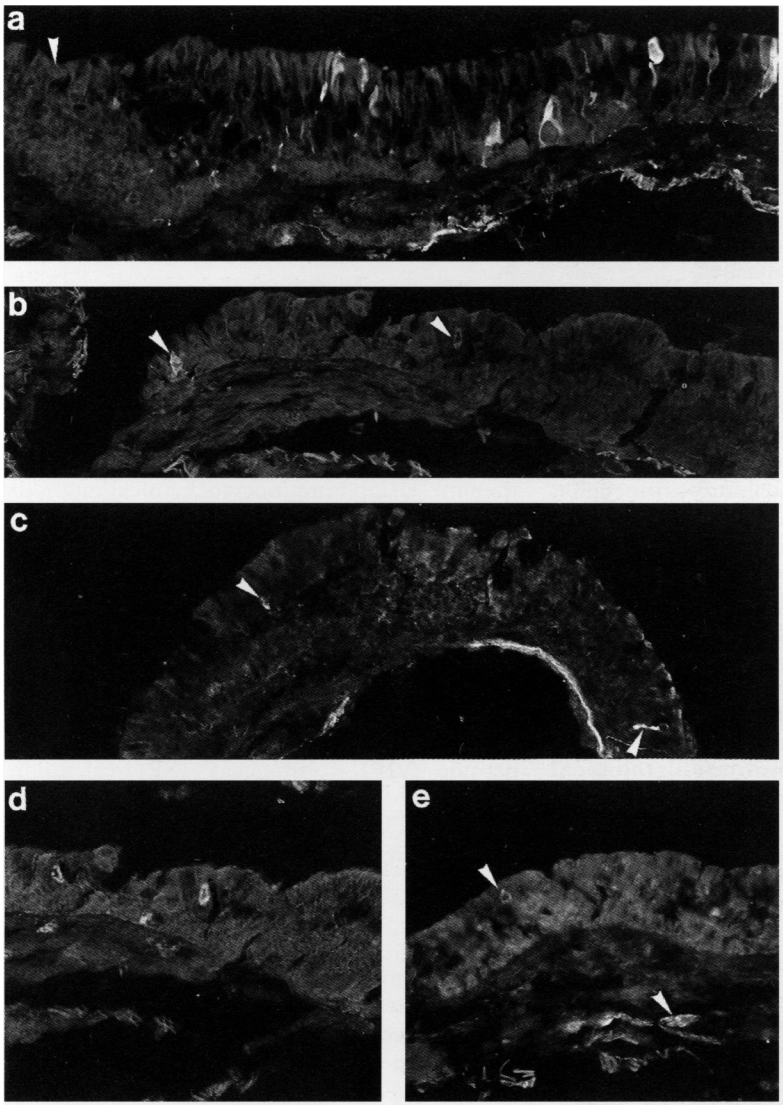

Figure 1 Immunofluorescence micrographs of bronchial wall with metaplastic epithelium in patients with chronic non-specific lung disease. (a) Numerous PGP immunoreactive neuroendocrine cells in normal epithelium; the metaplastic epithelium (arrow) is devoid of neuroendocrine cells. (b) Occasional weakly immunostained serotonin-containing neuroendocrine cells (arrows). (c) Cells containing chromogranin A and B in the metaplastic epithelium (arrows). (d) GRP immunoreactive cells. (e) Neuroendocrine cells showing weak CGRP immunoreactivity (arrow) in the epithelium as well as nerve fibres in the subepithelial layer (arrow). Original magnification ×250 reduced to 63% in origination.

were incubated in fluorescein isothiocyanate (FITC)-labelled swine antirabbit (or goat anti-mouse) IgG for 45 minutes at room temperature. After another rinsing in PBS the sections were mounted in phosphate buffered glycerin and examined by fluorescence microscopy. The specificity of the antisera used has been tested and the results presented elsewhere.[27]

Results

Examination of the biopsy specimens confirmed the presence of regions of non-metaplastic and metaplastic epithelium. In the non-metaplastic epithelium we found numerous PGP immunoreactive neuroendocrine cells (fig 1a). Most of the PGP positive neuroendocrine cells were immunoreactive for serotonin; only occasional serotonin positive neuroendocrine cells also stored CAB, CGRP, CT, and GRP. A few nerve fibres beneath the non-metaplastic epithelium showed weak PGP, VIP, CGRP, and SP immunoreactivity.

In regions with metaplastic epithelium we distinguished different distribution patterns of neuroendocrine cells and neuronal elements. Few, usually weakly stained, PGP, serotonin, CAB, GRP, CT and CGRP immunoreactive neuroendocrine cells were seen in these regions in six patients (fig 1b–e). The subepithelial layer beneath the metaplastic epithelium harboured fine PGP, CGRP, VIP, and SP immunoreactive nerve fibres (fig 1e); in some cases prominent PGP-containing nerve bundles were seen (fig 2a).

In areas of metaplastic epithelium in the other six patients many weakly stained PGP-containing neuroendocrine cells were seen. Most of them stored CAB (fig 2b). A sub-

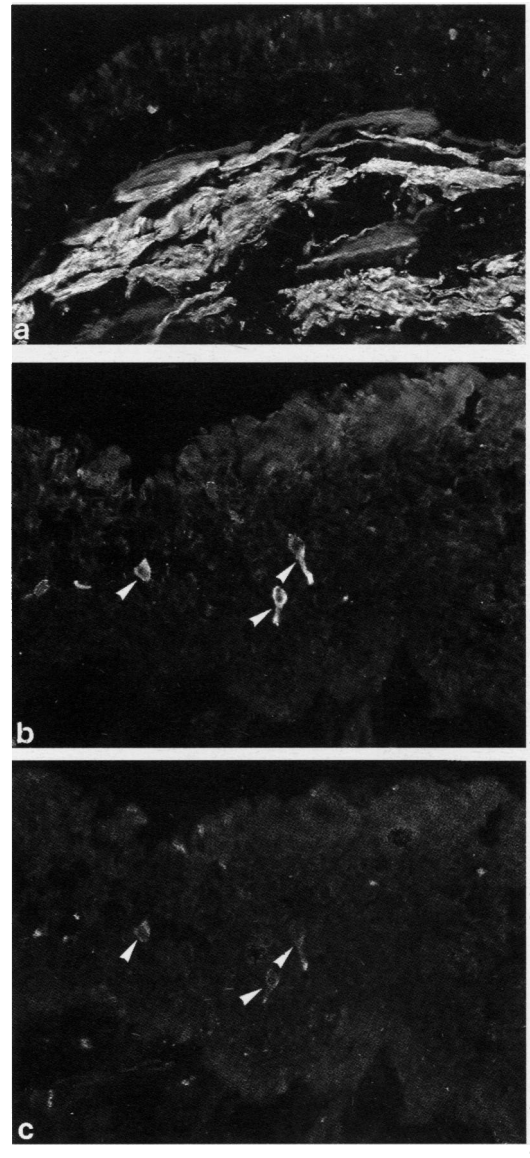

Figure 2 Immunofluorescence micrographs of bronchial wall with metaplastic epithelium in patients with chronic non-specific lung disease. (a) Prominent PGP-containing nerve bundles beneath the metaplastic epithelium occupying most of the subepithelial space. (b) and (c) Consecutive sections showing intensely CAB immunoreactive neuroendocrine cells (arrows) and serotonin immunoreactivity (c) in the same cells within metaplastic epithelium (arrows). Original magnification ×250 reduced to 57% in origination.

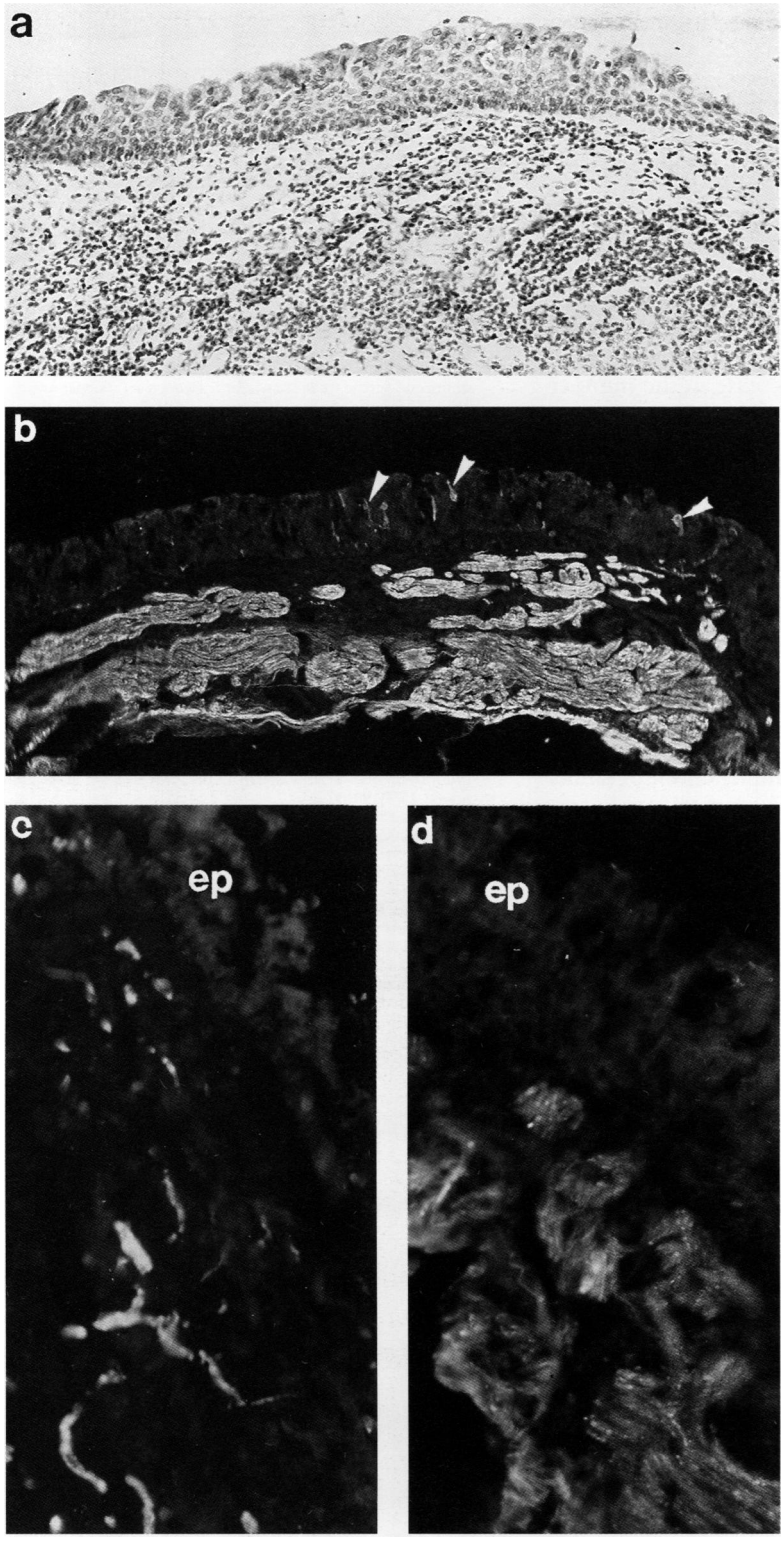

Figure 3 (a) Light and (b)–(d) immunofluorescence micrographs of sections from large bronchi of patients with chronic non-specific lung disease. (a) Characteristic bronchial metaplastic epithelium. (b) PGP-containing neuroendocrine cells in the metaplastic epithelium (arrows) and prominent immunoreactive nerve bundles in the subepithelial layer. (c) Numerous VIP-containing nerve fibres, some forming bundles beneath the epithelium (ep). (d) Numerous fine SP immunoreactive nerve fibres, sometimes forming nerve bundles in the subepithelium. Original magnification × 160 (a and b) and × 250 (c and d) reduced to 75% in origination.

population of the CAB immunoreactive neuroendocrine cells also displayed immunoreactivity for serotonin (fig 2c) in all six cases and for chromogranin A in three cases. Generally, the combined CAB antiserum detected higher numbers of neuroendocrine cells than the specific chromogranin A and the serotonin antibodies together. No neuroendocrine cells containing CT, CGRP, or GRP were found in the metaplastic epithelium of these patients. In those biopsy specimens where CAB immunoreactive cells were numerous, PGP-containing nerve bundles were prominent in the subepithelial layer beneath the metaplastic epithelium (fig 3a, b). Subpopulations of nerve fibres within these bundles contained VIP, SP, and CGRP (fig 3c, d). Aggregates of neuroendocrine cells (tumourlets) were not observed. We could not detect any correlation between the presence and abundance of neuroendocrine cells and neuronal elements in the bronchi and age, diagnosis, or duration of disease.

Discussion

Previous studies have indicated a relationship between neuroendocrine cells and nerve fibres in the respiratory tract.[8] In the present study we examined the presence and quantity of neuroendocrine cells and neuropeptide-containing nerves in the bronchial mucosa of patients with chronic non-specific lung disease displaying metaplastic and normal epithelium. The distribution pattern of neuroendocrine cells and neuronal elements and their marker and messenger content varied, not only between areas of normal and metaplastic epithelium, but also within the areas of metaplastic epithelium. It might be that these patterns change during the process of development of metaplasia. This possibility is supported by the presence of metaplastic epithelium in which only small numbers of neuroendocrine cells were seen and nerve bundles were inconspicuous in the subepithelial tissue. In other cases metaplastic epithelium possessed many CAB-containing neuroendocrine cells as well as prominent nerve bundles in the subepithelial layer. There are several reports on endocrine cell proliferation in diseased lungs.[9,10] An increased number of neuroendocrine cells in the lungs could be a physiological response to pulmonary injury or infection. Gosney[9] has suggested that this response might have a tendency to become disordered if stimuli which provoke it persist. He also described different stages of increases in the density of peptide-containing neuroendocrine cells during prolonged pathological processes in the lungs. The earliest detectable change was an increase of calcitonin-containing neuroendocrine cells; the next stage involved the appearance of ACTH, VIP, SP, and growth hormone immunoreactivity. It was suggested that proliferation of neuroendocrine cells might, if persistent, lead to the development of pulmonary tumourlets.[9] Thus, there may be a relationship between the stage of tissue disorder and the number of neuroendocrine cells and their chemical coding. We detected different patterns of changes in neuroendocrine cells and neuronal elements which did not depend on age, diagnosis, or even duration of illness. These findings suggest that there are other insults contributing to changes in neuropeptide-containing elements in the lungs.[10–12]

There was a strong association between numerous CAB-containing cells and prominent PGP-containing nerve bundles with numerous nerve fibres storing SP, CGRP, and VIP to form a dense network in the subepithelial layer beneath the metaplastic epithelium. This finding contrasts with the previous findings of a rather poor peptidergic innervation of the mucosa in human airways.[2 13] It is generally thought that chromogranins have intracellular roles in the package and/or processing of certain peptide hormones on neuropeptides and other granule constituents such as monoamines.[14] The increase in the number of nerve fibres, including prominent nerve bundles in the subepithelial layer, may be related to an increase in chromogranin-containing neuroendocrine cells. Although chromogranin A is known to appear in neuroendocrine cells in normal human bronchial epithelium,[15] chromogranin B has been found to occur mainly in bronchial carcinoids.[15 16]

Inflammation is thought to be a major factor responsible for epithelial metaplasia. The findings of neuroendocrine cell hyperplasia and a specific increase in CAB-containing neuroendocrine cells pose the question as to whether neuroendocrine cell-containing chromogranins play a part in the process of metaplasia, and whether subepithelial neuronal hyperplasia is also associated with this process.

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